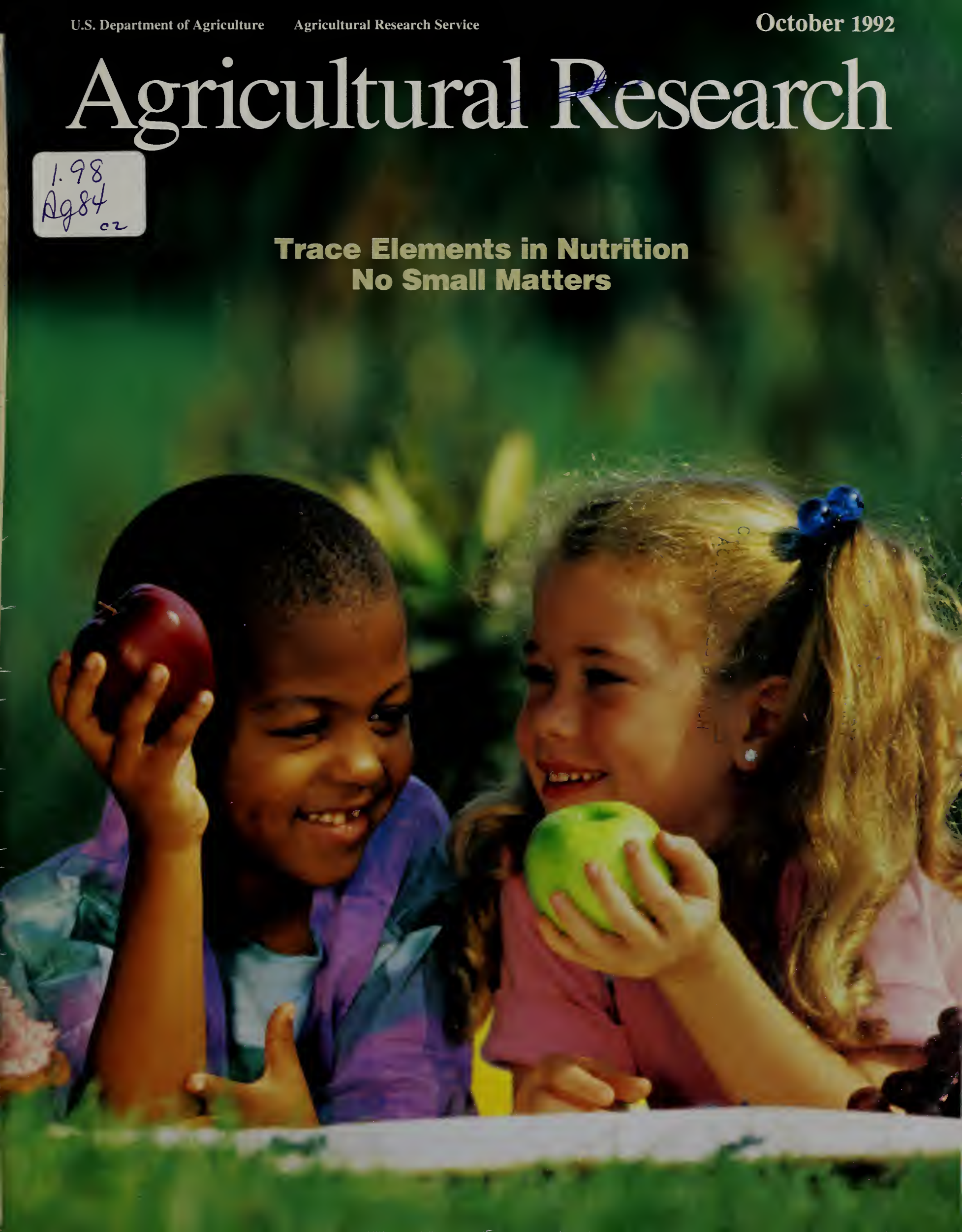
U.S. Department of Agriculture Agricultural Research Service October 1992

# Agricultural Research



## Trace Elements in Nutrition
## No Small Matters



## Speaking With One Voice

Growing evidence that our dietary habits play a major role in maintaining our health increasingly captures the interest of Americans. The subject sparks almost daily news reports on the latest findings of the health effects of particular foods or nutrients.

Unfortunately, this explosion of information has led to confusion in the minds of the public because research findings are often only fragments of fact that cannot yet show the full picture.

So it is not surprising that some nutrition findings are puzzling. Composition of test diets, study designs, and individual differences in test subjects all affect a study's outcome. That's why policymakers rely on the "preponderance of evidence"—not on a single study or the latest findings—to set guidelines for the public. And dietary guidelines are periodically revised as research produces new information.

Recognizing the public's need for solid, coherent information, the U.S. Departments of Agriculture (USDA) and Health and Human Services (DHHS)—the major federal players in nutrition and health matters—began "speaking with one voice" when they jointly published the eating guide titled "Dietary Guidelines for Americans," back in 1980. It has since been updated twice, most recently in 1990, by panels of experts.

Wisely, the first of the guide's seven recommendations is simply to eat a variety of foods.

Varying our diet allows us to get the full range of nutrients we need—even those science hasn't discovered yet. It also implies that there is no such thing as a "bad" food; only a poor diet. All foods—even high-fat fast foods, desserts, or candy bars—have some nutritional merit and can be fit sparingly into a healthful diet.

But the complete diet should include foods from the bread, fruit, vegetable, meat, and milk groups. USDA has developed a new Food Guide Pyramid graphic to visually illustrate the proportions of each food group that form a healthful diet—as science currently understands such a diet to be.

In another attempt to get the word out to consumers, DHHS' National Cancer Institute has joined forces with the Produce for Better Health Foundation, funded by a consortium of fruit and vegetable trade associations.

Together, they have launched a national campaign to make Americans more aware of the health benefits of fruits and vegetables—including their potential to lower the risk of cancer. NCI has adopted and revised for national distribution a brochure originally prepared by the State of California titled "5 a Day for Better Health." That's five servings of fruits and vegetables.

To identify the target audience, campaign managers reviewed major studies on Americans' attitudes about diet and nutrition. They also independently surveyed nearly 3,000 people about both their consumption of fruits and vegetables and their awareness of derived health benefits.

The findings show that only 8 percent of Americans think that they should eat five or more servings of fruits and vegetables each day as shown on the Food Guide Pyramid; 66 percent think one or two servings are ample. Nutritionists hope that the upcoming educational campaign will dramatically improve these numbers.

Congress also wants a careful and coordinated watch on the eating habits of Americans and how they relate to health. Two years ago this month, the National Nutrition Monitoring and Related Research Act was signed into law. It intends to strengthen nutrition monitoring by requiring the USDA and DHHS Secretaries "to prepare and implement a 10-year plan to assess the dietary and nutritional status of the U.S. population, to support research on, and development of, nutrition monitoring...."

These two departments conduct the two broadest nutrition and health surveys: USDA's Nationwide Food Consumption Survey and DHHS' National Health and Nutrition Examination Survey, or NHANES. But more than 40 other surveys have also evolved to meet the informational needs of federal agencies and other users.

A comprehensive 10-year plan has been developed and published in the Federal Register (October 26, 1991). It reflects broad input from other federal agencies, the public health community, and other users, such as scientific advisers to federal agencies, food and nutrition researchers, economists, the food industry, and academia.

Under the plan, ARS will collaborate with other federal agencies to support the diet/health connection. ARS' primary responsibilities will be:

- To improve measurements of human nutrient status—body levels and metabolic functions of vitamins, minerals, and other food constituents.
- To establish criteria for interpreting those measurements for subgroups of the population, such as infants or pregnant women.
- To define the requirement range for each age and gender group for nutrients considered to be current or potential health problems, for use in setting Recommended Dietary Allowances.
- To improve methods for analyzing foods for nutrient composition and develop quality controls that ensure data reliability
- To encourage the food industry to standardize food composition measurements and to voluntarily contribute their data for nutrition monitoring purposes.

**Jacqueline Dupont**
National Program Leader
for Human Nutrition

# Agricultural Research

**Cover: The Agricultural Research Service is committed to improving human nutritional status through research. Primary goals are to expand knowledge of the nutrients found in foods, their functions and interactions, and their biological availability, and to precisely define the nutrient needs of people of all ages.** Photo by Keith Weller. (K4819-1)

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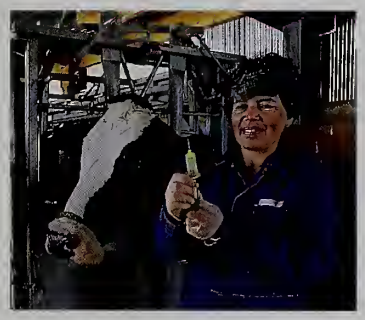

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# Good (for you) to the last drop!

## Researching Nutritional Trace Elements

The table manners practiced in the dining rooms of the Grand Forks Human Nutrition Research Center would curl the hair of etiquette aficionados.

Long-term guests and those who drop in for daily meals always lick their plates, bowls, and utensils, then spritz them with distilled water and drink the residue. But such manners—or lack of manners—are essential to the center's research.

Established by congressional mandate and opened in 1970, the center defines human requirements for minerals—particularly the trace elements—that amount to only thousandths or millionths of a gram each day.

So volunteers need to consume every last bit of their meals, which contain precisely measured amounts of these elements, down to the very last drop. This ensures that findings from dietary studies are based on accurate data.

Center director Forrest H. Nielsen says nearly 75 percent of the human and animal studies aim "to establish our need for various trace elements, the factors that affect that need, and the functions that are impaired if we don't get enough copper, manganese, or boron, for instance."

The other 25 to 30 percent of studies are "specifically designed to establish how much of a trace element we require to reach our genetic potential and maintain optimal health throughout life."

The National Academy of Science's Food and Nutrition Board uses these ARS data when it convenes a committee every 5 years or so to evaluate current Recommended Dietary Allowances (RDA) and Estimated Safe and Adequate Daily Dietary Intakes or to establish new ones.

The trouble is, says Nielsen, "very little research on trace elements is being done with human volunteers outside of ARS nutrition centers. Except for iron, iodine, and zinc, many in the nutrition community don't even think that trace elements are of much nutritional concern."

That explains why, of the 14 or so elements currently thought to be essential for humans, RDA's have been established for only these three, plus selenium. And selenium's addition to the 1989 RDA's was largely due to the efforts of an ARS scientist at the Beltsville (Maryland) Human Nutrition Research Center.

Estimated safe and adequate intake ranges have been set for another five elements—copper, manganese, fluoride, chromium, and molybdenum. But data are still too skimpy for official estimates of our requirements

Long-term guests and those who drop in for daily meals always lick their plates.

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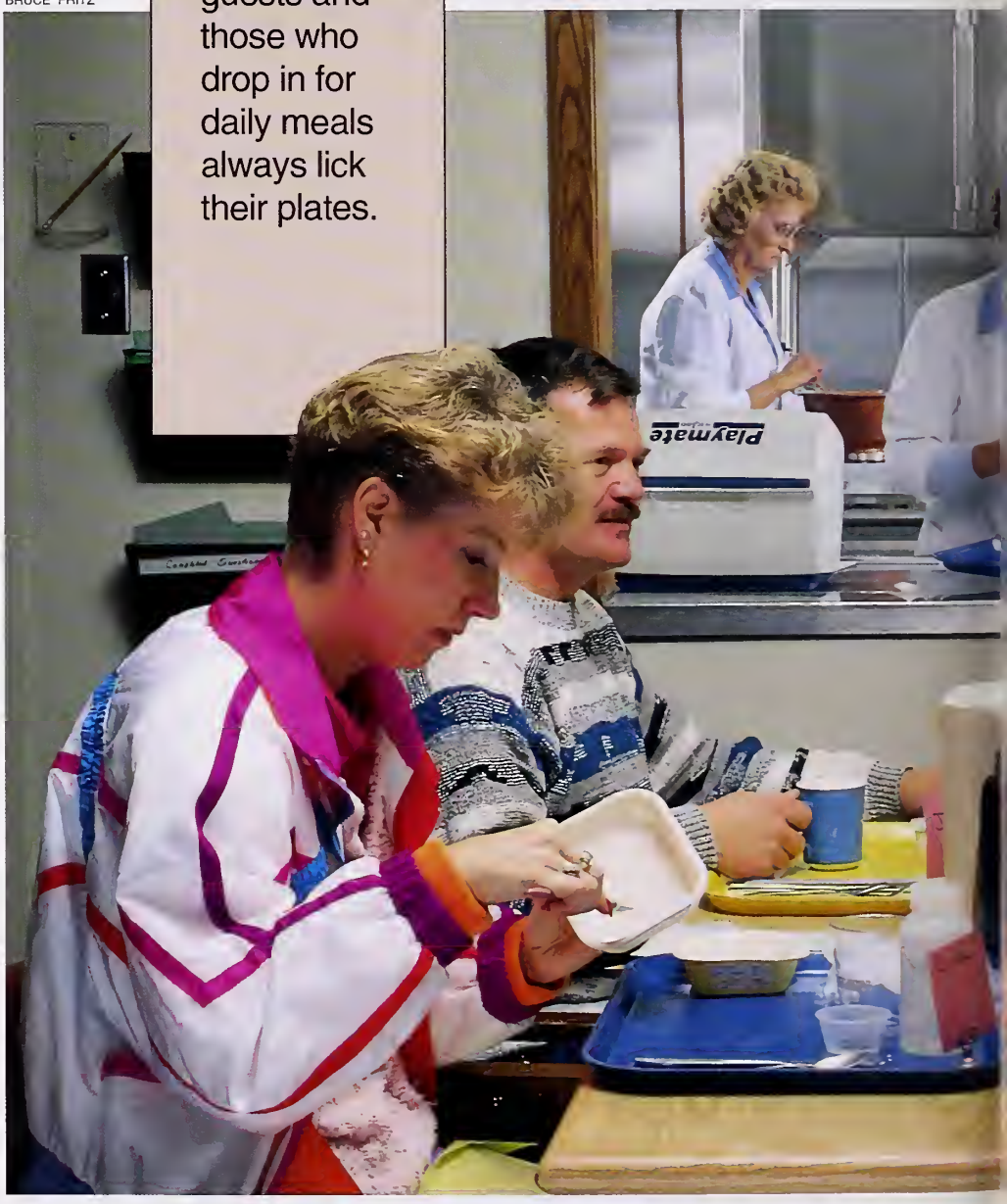

for silicon, vanadium, nickel, boron, and arsenic.

"Unless you identify problems caused by deficiencies," Nielsen asserts, "you'll never know if an element's important."

In the last few years, he and colleagues Curtiss D. Hunt and James E. Penland have put boron "on the map" for essentialness with dozens of animal studies and four human studies to date. Before that, boron was viewed as a traveler just passing through the body with no known metabolic function.

Grand Forks researchers are also conducting animal studies to show the functions and consequences of deficiencies in silicon, vanadium, nickel, and arsenic. That's right, arsenic!

"All trace elements are toxic to one degree or another when taken in excessive doses," says Nielsen. "But it's virtually impossible to get toxic levels of any element through uncontaminated food."

Before the turn of the century, iron and iodine were the only elements recognized as essential for humans. Most of the others, which are sometimes called trace metals, were "discovered" after 1950.

No doubt, new elements will show up with further research. And newly recognized functions for established trace elements continually crop up. So the Grand Forks staff has its research cut out for it well into the next century.

**Volunteers for nutrition studies at the Grand Forks Human Nutrition Research Center eat their meals while technicians (behind counter) weigh food and check trays to make sure every drop and crumb has been eaten. (K4764-9)**

### Data for setting the RDA's

"All of the Grand Forks center's human studies contribute useful information for setting RDA's—but some more than others," says Phyllis E. Johnson, who oversaw research on trace element biological availability and absorption there until last fall. "They have also contributed significantly to the new requirements to be published by the World Health Organization."

Now associate director of ARS' Pacific West Area headquartered in Albany, California, Johnson still has a hand in studies she started while at the center.

RDA's are largely based on the amount of an element people need to stay in balance. That's how much we need to consume each day to replace what is lost through the urine, feces, sweat, menstrual flow, and seminal fluid. And it changes throughout our lifetimes.

But because people don't absorb all of the trace elements that are in their

foods, researchers also have to determine what percentage is absorbed for the RDA committee to arrive at a recommended intake level. And this absorption rate differs for each element.

Arriving at such figures requires hundreds of precise measurements and a lot of sophisticated number crunching.

In a 2-month study of zinc balance, nutritionist Janet R. Hunt found that men and women lost an average 2 milligrams of zinc a day through urine and feces. But they absorbed only 25 percent, on average, of the zinc present in their diets. This means they would have to consume four times the 2-mg loss, or 8 mg daily, to ensure absorption of enough to replace that loss.

Hunt says she began the study because the 1980 RDA's based the zinc requirement on the need to replace a 6-mg daily loss at an absorption rate of 40 percent. But later studies pointed to both a much lower daily loss and a much lower absorption rate.

While Hunt's study was under way, the 1989 RDA's were published. She says, "It based the zinc requirement on a 20-percent absorption rate and a loss of 2.5 mg per day. My data support it."

She adds that absorption rates can vary widely, depending on many factors, so the small difference between her rate and the revised RDA basis is not surprising. And the loss of zinc in sweat, which she did not measure, could account for the slightly higher daily loss used by the RDA committee. "I'm much happier with the revised bases than I was when I started this study," she says.

Hunt says that the changed bases for setting the 1989 zinc RDA didn't alter the recommended amount for men because the absorption rate dropped along with the quantity needed for daily replacement. It remains at 15 mg for men. But the committee did reduce women's RDA to 12 mg because of their smaller body size.

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**Studies with human volunteers contribute information that will be used in setting trace element RDA's. (K4765-10)**

The RDA's are actually higher than the bases call for, Hunt explains, because the data from studies are average values. The RDA's include an added safety factor to protect individuals with the highest requirements.

The committee also considers how much of an element people are already consuming in recommending daily intakes, says Hunt. "The reasoning is, if people are generally healthy, their intakes must be meeting their needs."

### Assessing Trace Element Needs

There is no RDA for copper yet. But a study led by Johnson on the effects of age and gender on copper requirements should help move us closer to one. And the findings indicate that women need a little less copper than men—at least up to age 60—because of their smaller size.

Before her study, she says, "there were no data showing whether men and women differ in copper absorption, rate of loss, or basic requirements. And studies on the effect of aging on copper status were limited."

So Johnson measured the copper intakes of 127 men and women from 20 to 83 years old living in the Grand Forks area. She also assessed copper absorption and loss by giving each volunteer a harmless dose of radioactive copper and measuring how long it stayed in the body.

The men consumed about 1.3 mg of copper daily, she says. The women took in 1.1 mg. "My feeling is that these intakes are typical and probably sufficient." The estimated safe and adequate intake is 1.5 to 3 mg per day for both men and women, but most people consume less copper than that.

Johnson notes that the women consumed less copper than the men but compensated by absorbing a higher percentage of their intake. So, pound for pound, both genders absorbed the same amount. "This suggests that women have a lower requirement for dietary copper intake than men," she says, because they generally weigh less.

After age 60, however, the trends seem to reverse. Johnson says that some indicators of copper status slipped in the older women. And the older men absorbed slightly more copper from their diets than the older women. But there were too few older volunteers in the study to draw any statistically sound conclusions, says Johnson, other than that there is a need for a study with more older volunteers.

It's long been known that iron has a profound effect on our energy levels: Blood hemoglobin needs iron to

**Copper**: Used in the body to aid iron absorption, in hemoglobin, and for cardiovascular health. It is most plentiful in liver, oysters, lobster, nuts, seeds, olives (green), soy flour, wheat bran, wheat germ, dark chocolate, and dried peas.

deliver oxygen to body cells. But other trace elements increasingly appear to affect energy metabolism in more subtle ways.

Physiologist Henry C. Lukaski began a series of rat studies to demonstrate the effects of zinc on thyroid function—the primary regulator of energy metabolism. Other investigators, he says, had reported that a low-zinc intake slowed people's basal metabolic rate and reduced blood levels of thyroid hormones.

Since the thyroid doesn't operate in a vacuum, Lukaski explains, he and collaborators looked at the biochemical pathway that stimulates the gland to release its hormone, known as $T_4$. Then he and center colleagues looked at the pathway that regulates the conversion of $T_4$ in the blood to its more active form, $T_3$.

"There are zinc-dependent enzymes that regulate the synthesis of precursors of TRH (thyrotropin-releasing hormone)," he says, "but no one has looked at the effects of zinc deficiency on TRH production." Synthesized in and around the brain's hypothalamus, TRH stimulates the pituitary gland to release TSH (thyroid-stimulating hormone). TSH, in turn, signals the thyroid gland to release $T_4$.

Lukaski collaborated with researchers at the Veterans Administration's Wadsworth Medical Center in Los Angeles on investigating the brain pathway. They found that "the rats fed a no-zinc diet couldn't make the TRH precursors," he says. "And precursor levels were reduced in the animals that got only half the amount of zinc recommended for test rats."

He suspects that inadequate dietary zinc is blunting the activity of the zinc-dependent enzymes that regulate synthesis of these precursors. But that's a question for a future study.

Zinc-poor diets also have an effect at the other end of the thyroid hormone pathway. Rats fed either a no-zinc diet or a reduced-zinc diet had significantly lower blood levels of $T_4$ and $T_3$ as well as TSH. The more deficient the diet, Lukaski notes, the greater the reduction in these hormones.

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**Food, precisely measured to the nearest hundredth of a gram, is placed on study volunteers' trays by dietician Lori Matthys. Each portion is adjusted for the individual's calorie levels on a 3-day rotating cycle menu. (K4765-2)**

And a big dip in these hormones can have a chilling effect. When the rats that got a no-zinc diet were put in a cold room several degrees above freezing, they couldn't maintain their core body temperature.

They produced more norepinephrine, or adrenaline, in an attempt to stoke their metabolic furnaces. But their thyroid hormone levels were just too low to maintain body heat.

Boron is another trace element that increasingly appears to be involved in energy metabolism. But its effects are obvious only when test animals are under stress, says Grand Forks anatomist Curtiss D. Hunt, who has been studying these effects for nearly a decade.

For instance, vitamin D deficiency causes several metabolic abnormalities in chickens, including elevated levels of blood glucose and triglycerides as well as pyruvate—a primary product of glucose metabolism. Adding boron to the chicks' diets markedly decreased those elevated levels.

Boron also reduced blood pyruvate levels in vitamin D-deprived rats, Hunt notes. This could mean that boron increases the rate at which the rats recycle energy metabolites. Or it could mean that the rats had less glucose to metabolize, which seemed to be the case. Their glucose levels tended to be lower than those in the control animals, he says, but not significantly.

Now, Hunt is finding in a new series of studies that he can alter a biochemical indicator of muscle function in exercising rats simply by changing the level of boron in their diets.

"I'm convinced that the amounts of boron found in a healthful diet affect energy metabolism," he says. "It appears to increase the rate at which animals burn fuel and perhaps the efficiency by which it is burned. But we don't yet know the mechanism."

He and Nielsen are also convinced that boron affects the way we use minerals—particularly, some minerals important to healthy bones: calcium, magnesium, and copper. Human studies led by Nielsen indicate that boron helps stem the loss or improves the absorption or use of these minerals. And animal studies done by Hunt show that boron helps overcome bone abnormalities and altered gait that result from raising chicks on vitamin D-deficient diets.

**Boron:** Used to maintain healthy bone structure. It is found in noncitrus fruits such as apples, pears, peaches, grapes, and cherries; nuts, dried fruits, broccoli, carrots, green beans; dried peas, beans, lentils, and other legumes.

Hunt says the reason boron's effects have gone unnoticed is that most

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**Physiologist Henry Lukaski evaluates the effect of changes in dietary trace elements on bone and muscle mass with dual energy x-ray absorptiometry. (K4763-1)**

commercial feeds for test animals are quite high in the element. A typical rat chow contains about 12 parts per million, whereas he sees positive effects beginning at only 1.4 ppm. And commercial chick starter contains alfalfa, which is naturally high in boron.

Plant-based foods are much higher in boron than animal foods, says Hunt. He has analyzed dozens of foods common in the human diet and found the richest boron sources to be apples, pears, grapes, and the juices of these fruits.

### Too Little Copper—Hard on the Heart?

Copper is one trace element that may have giant implications for public health. More than 20 years ago, Grand Forks physician and research leader Leslie M. Klevay raised rats' plasma cholesterol by feeding them a low-copper diet.

He hypothesized that long-term, inadequate dietary intake of copper is a major factor in the prevalence of heart disease—the number one cause of death in the United States.

Klevay has amassed a veritable library of epidemiological studies on heart disease and animal studies on the effects of copper deficiency. So far, he says he has identified "more than 60 similarities between animals deficient in copper and people with heart disease." These include such major risk factors as elevated blood pressure, blood glucose, and cholesterol levels; abnormal heart rhythms; and differences between male and female responses to low copper.

One similarity was recently discovered in Klevay's lab by postdoctoral fellow Sean M. Lynch, who is now at the Harvard School of Public Health. Lynch found that copper-deficient mice took 2.5 times longer to dissolve blood clots than mice that got adequate copper in their feed.

Heart disease patients also take longer to dissolve clots when assessed by the same test done on the mice—the euglobulin clot lysis test (ECLT), says Klevay.

Tiny blood clots are part of the plaque-forming debris that accumulates in arteries, gradually narrowing the vessels and reducing blood flow. If the ability to dissolve a clot is impaired, the clot thickens—and so does the plaque.

Klevay points out that copper is a component of enzymes the body uses to make all three types of connective tissue

found in arteries. It is also a component of at least three enzymes that protect body tissues, including arteries, from damage by oxygen free radicals, says Jack T. Saari, a physiologist.

In earlier collaboration with researchers at the Veterans Administration's Medical Center in Tucson and at the University of North Dakota Medical School in Grand Forks, Saari found that copper-deficient rats are more susceptible to oxidative damage. And they were protected by adding antioxidants to their feed. [For more about oxygen free radicals, see "Vitamin E Is for Exercise," *Agricultural Research,* September 1992, p. 14.]

Now he is finding that copper deficiency causes changes in heart and arterial cells that further point to its role in heart disease.

Over the last dozen years, Saari explains, scientists have learned that the cells lining all blood vessels are not passive. When stimulated by certain bloodborne chemicals, these endothelial cells release substances that cause the adjacent smooth muscle cells to either relax or contract.

When the muscle cells are signaled to relax, blood pressure goes down. But copper deficiency decreases release of endothelial-derived relaxing factor (EDRF) in test rats' aortas—the largest artery in both humans and animals, says Saari.

Another study with researchers at the University of Louisville produced the same results for smaller blood vessels, known as arterioles.

Saari says: "We think this is the mechanism by which copper deficiency raises blood pressure in adult rats."

Although people consume more copper than the animal diets contained, a large majority of Americans don't get the estimated safe and adequate daily intake of 1.5 to 3 mg.

According to USDA's 1987-88 food consumption survey data to be published soon, U.S. men average 1.2 to 1.3 mg of copper daily from their diets; women average 0.9 to 1.0 mg. Over many years, these marginal intakes may fail to maintain the integrity of arteries and protect them from damaging oxygen free radicals.

What's more, too much of the sugar fructose can magnify the effects of a marginal copper intake, according to studies at the ARS Beltsville center and now at Grand Forks.

Nielsen and chemist David Milne looked for signs of oxidative stress in a carefully controlled 7-month study of six men during which their copper intake was reduced to 0.6 mg per day and fructose supplied 20 percent of their calories.

Americans typically derive 10 to 14 percent of their calories from fructose, which constitutes half of table sugar (sucrose) and close to half of high-fructose corn sweeteners, explains Milne. People who consume a lot of nondiet soft drinks can easily get more than 15 percent.

While extra fructose did not reduce copper absorption, it significantly decreased levels of two copper-containing enzymes that function as antioxidants when the men got only 0.6 mg of copper per day.

Also, the low-copper intake by itself caused the men to produce more of an antioxidant that does not contain copper to take up the slack. Glutathione levels went up during the low-copper periods, regardless of whether the diets contained fructose or the control carbohydrate, a starch, says Milne.

He concludes that "fructose may increase the potential for tissue damage by oxygen free radicals during short-term copper deprivation."

He notes, however, that there was so much variability among the six men that factors other than dietary copper—for example, genetic makeup, previous body levels of copper, and exposure to oxidant stress—must have influenced their response to short-term deprivation.

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**To determine the effect of weight loss on trace mineral metabolism and energy expenditure, physiologist Henry Lukaski calculates a volunteer's oxygen intake and carbon dioxide output. (K4768-3)**

"We're getting all kinds of evidence that copper deficiency has pathological consequences," says Nielsen. "I think low copper intakes typical in the United States and in other industrialized countries can lead to problems, particularly in older people."

Possibly, just one extra milligram of copper each day could put a big dent in the incidence of heart disease. But the burden of proof falls on the Grand Forks center, where many of the human copper studies are being done.

Future studies may also show the potential of zinc, magnesium, boron, or other obscure trace elements to keep us healthier well into old age. The work at Grand Forks and other ARS centers is demystifying their functions and proving their importance. This new knowledge should inspire others in the nutrition community to help define human requirements for all trace elements.—By **Judy McBride,** ARS.

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**Chemist Phyllis Johnson loads samples into a mass spectrometer to measure copper isotope ratios. (K4761-1)**

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**In a copper study, technician Kay Johnson measures cells in blood samples as nutritionist Forrest Nielsen checks data for changes. (K4767-1)**

## Trace Elements' Psychological Effects

While other Grand Forks researchers concern themselves with the effects of dietary trace elements on biological functions, James G. Penland delves into the psychological consequences. He may be the only psychologist in the United States studying trace element nutrition.

Bolstered by electroencephalographs that record brain wave activity, by motor function tests, and by a battery of psychological questionnaires, he is finding evidence that trace elements can have serious effects on our brain, behavior, and mood.

For instance, when people ate foods deficient in boron, their brain waves changed in ways suggesting a less-alert state, and their reaction times to visual cues were slower.

In other studies, low intakes of copper or iron or high intakes of aluminum (common in many antacids) interfered with good-quality sleep. And extra calcium relieved many of the physiologic and psychological symptoms women experience before and during menstruation.

### Dieting and PMS

Not all of Penland's findings deal with minerals, however. In a recent study of 14 obese women on a weight-loss regimen, he showed that the women experienced fewer menstrual-related symptoms after researchers cut their normal calorie intake in half and put them through a vigorous exercise program.

Penland had the women indicate the presence and severity of 47 symptoms listed on a standard menstrual distress questionnaire after each phase of the study. By the end of the study, when calorie intake was lowest and aerobic workouts were hardest, they reported fewer problems with PMS-type mood swings, less antisocial or inefficient behavior, better concentration, and less water retention during menstruation, he says.

In fact, he notes, "Their scores for these symptoms were 40 percent below those given before they cut calories and began exercising." And the evidence from the psychological tests is strengthened by the fact that the women's blood levels of monoamine oxidase dropped when they were dieting and exercising. This enzyme has been suggested as a factor in premenstrual tension.

### What About Pain?

Penland began wondering if mineral intakes could affect pain levels after women reported less pain during menstruation with extra calcium in their diets. As in any hospital, nurses at the Grand Forks center's metabolic unit record each and every medication they dispense to volunteers who live at the center during controlled studies.

So he analyzed data from eight previous studies to see if volunteers requested pain pills (common analgesics such as Tylenol, Advil, Sudafed, and Motrin) more or less frequently as mineral intakes changed.

Luckily, four of the studies were done with men and four with women, half of whom were young and the rest past menopause. He excluded any requests associated with a specific injury, infection, or chronic illness, such as arthritis, using only requests for general aches and pains.

Sure enough: "In five of the eight studies," he says, "there is a significant effect of diet on the percentage of days the volunteers requested pain medication. Requests were two to three times higher when the diets were most restrictive."

Low copper intakes, a combination of low calcium and low manganese intakes, or a dramatic cut in calorie intake triggered either more pain or more awareness of pain, says Penland. "The diet seems to be related to the perception of pain, and more than one mineral seems to be involved."

But, he cautions, researchers will have to repeat these findings before the evidence is convincing enough for recommendations for dietary changes to alleviate pain.

Penland also looked for differences in pain perception relative to menstrual cycle phase in the two studies with younger women, because women typically take a lot more pain medication when menstruating. These were the weight-loss study with a 50-percent cut in the women's normal calorie intakes and another involving high versus low intakes of both calcium and manganese.

The result: "When the diet was low in essential nutrients or calories were severely restricted," he says, "the women made almost as many requests for pain medication during the nonmenstrual phases as they did during menstruation."—By **Judy McBride,** ARS.

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**In front of a mountain of phosphogypsum, Michael Lloyd (facing camera), research director for chemical processing at the Florida Institute of Phosphate Research, discusses uses for the product with a mining company representative. (K4153-11)**

# What a Waste!

## Phosphogypsum Enriches the Soil

The huge, unsightly mounds can stretch across hundreds of acres. Some covered, some uncovered—these mountainous stacks can be an eyesore on the flat, sandy Florida skyline. Whitish gray in color, with a crusty surface, they look like massive heaps of table salt that tower up to 200 feet high.

This is Florida's stockpile of phosphogypsum. More than 600 million tons of it are already on the ground and an additional 30 million tons accumulate yearly.

When mined phosphate rock is treated with sulfuric acid, the result is phosphoric acid, which is used to make fertilizer. Phosphogypsum (PG) is a byproduct of the process.

Phosphogypsum has been used in construction materials and as a base in building roads. Its largest value, however, might be agricultural.

"This material could be used beneficially as a soil amendment," says Stanley Nemec. "We applied it for 3 years, at up to a ton per acre, on three orange groves with good results."

Not only did the PG improve overall tree health, but it also increased the calcium and manganese levels in the fruit juice and calcium levels in leaves and bark. "Citrus is a heavy user of calcium, needing more of it than many other plants," says Nemec.

An ARS plant pathologist with the U.S. Horticultural Research Laboratory in Orlando, Florida, Nemec collaborated with University of Florida scientists Don Myhre and Ron Sonada.

"One finding was surprising: PG can probably reduce foot rot, a major citrus disease in Florida that causes cankers on tree trunks and rots the roots," Nemec says.

The high concentration of calcium in PG apparently strengthens tree cell walls against invasion by this fungus-borne disease, he says.

"This unexploited source of calcium could also help avoid drought stress by facilitating deeper penetration of calcium—and consequently, of roots—into the acid subsoils of the Southeast," says Doral Kemper, ARS national program leader for soil management.

RICHARD NOWITZ

But the use of phosphogypsum has been controversial because it contains a small amount of radium. Therefore, until consumers understand that they are not endangered by crops grown on PG-treated soil, most citrus growers hesitate to use PG.

The U.S. Environmental Protection Agency has also been cautious. Under a section of the Clean Air Act, it banned the use of phosphogypsum in April 1990 but issued a waiver for agricultural use through the growing season that ended October 1, 1991.

Then EPA issued a final ruling in the June 3, 1992, Federal Register, says Craig Conklin, health physicist with EPA's Office of Radiation Programs.

"The new ruling explicitly permits the controlled use of phosphogypsum in both agriculture and research and development," he says.

According to Conklin, "controlled use" means that when PG is used for agriculture, the average radium-226 level must not exceed 10 pCi/gram. This amount translates to 0.01 parts per billion, or the equivalent of less than one drop of water in two Olympic-sized swimming pools. Anyone who distributes PG must certify its radium-226 level in writing.

"We tested the fruit from our 3-year experiment with soil enriched with phosphogypsum and found no increase in levels of radium 226 from normal levels in fruit grown in untreated plots," Nemec reports.

Since Florida has set up the Institute of Phosphate Research to look for ways to use this abundant waste product, Nemec hopes his collaborative research results will provide new potential PG uses and complement the Institute's goal. Michael Lloyd, research director for the Institute, supports Nemec's studies.

## Soil Benefits

"We've been trying to tell people about the benefits of PG for years. Phosphogypsum is a superior amendment for many soil types and is an excellent fertilizer source of sulfur and calcium." Lloyd says. Any soil that tends to surface-harden and resist water, he contends, would benefit from PG. "It creates a more porous texture that soaks up rainfall."

Although other sources of sulfur are available, Lloyd says PG is ideal because it is only slightly soluble in water and therefore longer-lasting in the soil. Since the sulfur in PG is present in the form of sulfate, it can be absorbed directly by the plant: sulfur from most other sources must first be converted to the sulfate form before the plant can use it.

And, being neutral in soil reactions, sulfur from PG doesn't change soil acidity.

"This would be a real boon for citrus growers. They now use limestone, which raises the soil pH and may be a factor causing plant nutrient problems," Nemec says.

Lloyd explains that gypsum has also been used to reduce toxic elements in the soil. When present in heavy concentrations, almost any soil element can be toxic to plants.

Abnormal levels may occur naturally or may be introduced by the application of fertilizers, insecticides, or fungicides. In some cases, PG can inactivate toxic soil elements or, in other cases, mobilize them so they are easily leached from the soil.

Research is under way to determine how different elements will be affected by PG additions.

"Farmers have used phosphogypsum as a soil amendment for years and have come to rely on it," says Ron Phillips of the Fertilizer Institute, Washington, D.C.

Peanut grower Hubert Dollar of Bainbridge, Georgia, is one of those farmers. "I use from 1,000 to 2,000 pounds of phosphogypsum per acre for each peanut crop. To mature properly, peanuts need lots of calcium. PG is the cheapest source," Dollar says.

He's been adding PG to his soil in similar amounts for about 20 years, and his father did the same for many years. Last year, Dollar had tests run on his soil, peanut plants, and peanuts. Result? "No sign of any radium," he says.

Although the growing piles of phosphogypsum may pose an environmental problem where they are stored, use in the agricultural sector could reduce the stockpiles and benefit growers.—By **Doris Stanley,** ARS.

*Stanley Nemec is at the U.S. Horticultural Research Laboratory, USDA-ARS, 2120 Camden Road, Orlando, FL 32803. Phone (407) 897-7300, fax number (407) 897-7309.* ◆

# Making Peach Trees More Cold Hardy

Their simplicity is beautiful. But a lot of nature's complexities go into making those fragile, delicately scented blossoms that decorate peach trees in the early spring.

Had the trees not earlier experienced a cold, dormant period, the blossoms would never have appeared. Nor could they exist if temperatures had been too cold during that dormant period.

As the seasons change, fruit trees acclimate themselves to changing temperatures, building their cold tolerance as winter approaches. "Deciduous trees require a certain amount of chilling to produce flowers and then fruit," explains ARS plant physiologist Michael Wisniewski.

But although a fruit tree prepares itself for seasonal temperature changes, a harsh winter can damage buds, bark, and wood in the trunk and limbs. Trees can even be killed by severe cold spells. And in the spring, blossoms are particularly vulnerable to late frosts. Any of these can spell economic disaster for fruit growers.

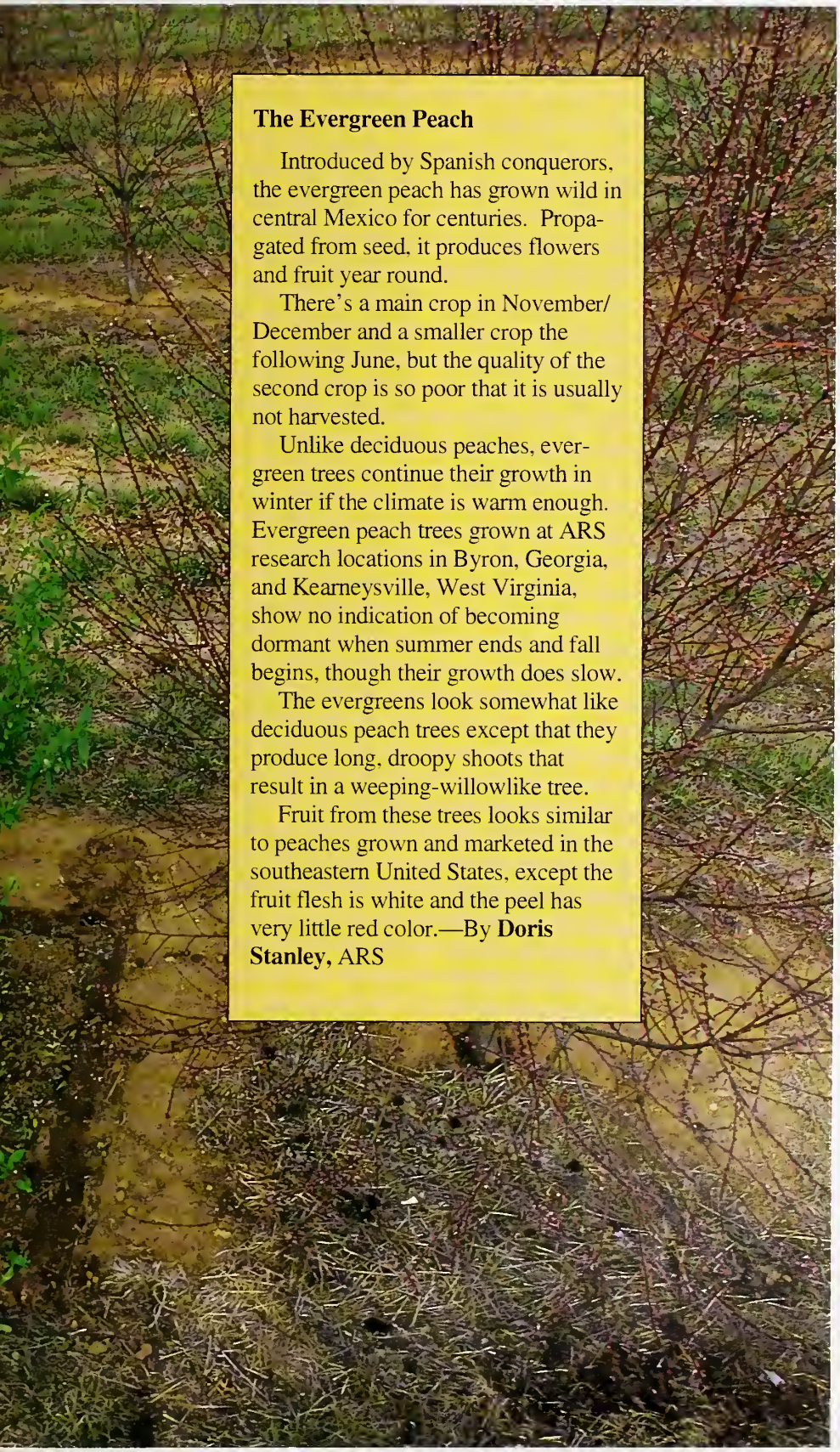
For several years, Wisniewski and horticulturist Ralph Scorza have been studying cold tolerance at the ARS Appalachian Fruit Research Station in Kearneysville, West Virginia.

Their perseverance paid off recently with a new theory of how plants keep themselves from freezing.

Working with scientists in the United States and Mexico, Wisniewski and Scorza identified a single gene that appears to control dormancy. And they've found a new protein that accumulates in tree bark as the tree slips into dormancy.

"We knew that living cells in woody portions of a tree avoid freezing by deep supercooling," Wisniewski says. "What this means is that the water inside each cell cools to below the normal freezing point without forming ice crystals."

SCOTT BAUER



### The Evergreen Peach

Introduced by Spanish conquerors, the evergreen peach has grown wild in central Mexico for centuries. Propagated from seed, it produces flowers and fruit year round.

There's a main crop in November/December and a smaller crop the following June, but the quality of the second crop is so poor that it is usually not harvested.

Unlike deciduous peaches, evergreen trees continue their growth in winter if the climate is warm enough. Evergreen peach trees grown at ARS research locations in Byron, Georgia, and Kearneysville, West Virginia, show no indication of becoming dormant when summer ends and fall begins, though their growth does slow.

The evergreens look somewhat like deciduous peach trees except that they produce long, droopy shoots that result in a weeping-willowlike tree.

Fruit from these trees looks similar to peaches grown and marketed in the southeastern United States, except the fruit flesh is white and the peel has very little red color.—By **Doris Stanley,** ARS

In order to accomplish supercooling, the cells must maintain their fluid contents free of ice-nucleating agents and prevent water or ice crystals from entering them from spaces between the cells.

"We discovered that the pit membrane—a portion of the cell wall through which water and nutrients flow—is rich in pectins that form a barrier to keep ice from entering into the cell when temperatures drop," he says.

According to Wisniewski's research, the type, amount, and degree of cross-linking, or interconnecting, of these pectins may determine the size of the pores of the pit membrane. And the pore size determines the cell's permeability to water.

Plant cell walls are made of cellulose and hemicellulose organized into long, rodlike units called microfibrils that are the building blocks of the cell wall. Pores are spaces between the microfibrils that allow substances to move into and out of the cell.

Pectins, forming layers along the outside of the microfibrils, act as a glue. This makes the pores even smaller—almost nonexistent—thereby substantially decreasing cell wall permeability.

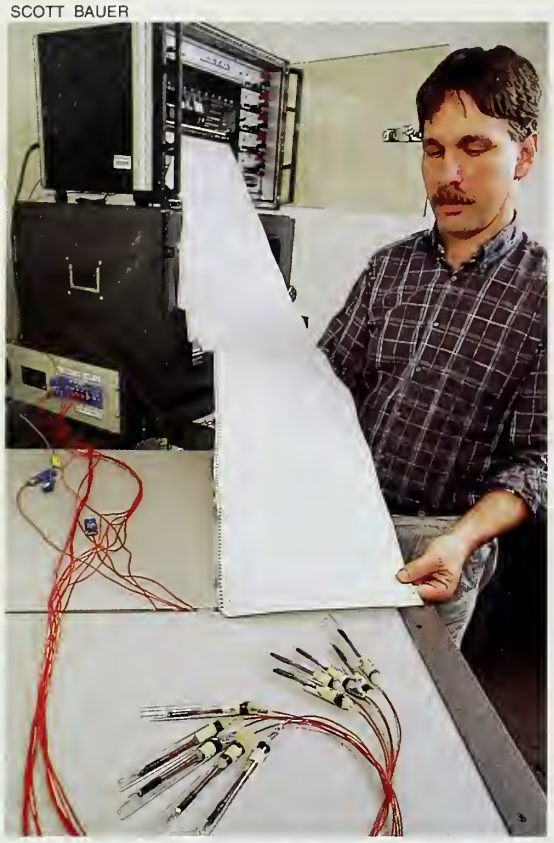
ARS scientists have been able to change the way plant tissue responds to cold temperatures by modifying the structure of the pectins in the cell wall.

"With this information, we may be able to breed trees for increased cold hardiness at critical times of the year," Wisniewski says.

Previous research indicated that cell wall porosity and permeability were key factors in supercooling, but until now there was no direct evidence that could support this theory.

**Plant physiologist Michael Wisniewski (right) and horticulturist Ralph Scorza collect shoots from an evergreen peach tree for protein analysis and evaluation of cold-hardiness. (K4684-11)**

SCOTT BAUER



**By using data from thermocouples that sense changes caused by heat, technician Glen Davis analyzes peach xylem tissues after freezing. (K4687-7)**

"Our knowledge of the genetic regulation of cold-hardiness in woody plants is limited," Wisniewski says. "The time when plants become dormant happens to be the time when they are also most cold-hardy. The simultaneous occurrence of these two physiological events makes it more difficult to figure out what's going on genetically."

However, this may be easier now that Scorza and his coworkers have discovered a single gene that appears to control dormancy in peach trees.

The gene, named "EVG" for evergreen, was first found in peach trees in Mexico that never go dormant. Trees recessive for EVG require little or no cold-induced dormancy to blossom and produce fruit, "unlike the peach trees we're familiar with," Scorza says.

"We crossed the evergreen peach with a deciduous peach. Then we grew second-generation (F2) hybrid trees in Mexico, Florida, Georgia, and West Virginia to test their dormancy requirement and cold-hardiness."

Scorza's coworkers in the evergreen peach research included W.R. Okie with ARS in Byron, Georgia, J. Rodriguez-A. with the Colegio de Postgraduados in Chapingo, Mexico, and W.B. Sherman with the University of Florida at Gainesville.

The close cooperation between U.S. and Mexican scientists and among researchers at the four locations made it possible to identify this important gene. This type of cooperative scientific exchange is necessary when working with new, unique fruit germplasm, Scorza says.

As research progresses from the physiological to the molecular level, other genes may be identified. If so, Scorza foresees plant breeders genetically modifying plants to produce more cold-hardy varieties.

"The advantage of gene transfer is that once a gene is isolated, it can be manipulated and possibly inserted into any variety," he says.

At this time, regeneration and transformation rates of fruit crops are low compared with other species, but once a transgenic fruit or nut variety has been produced and tested, it can be multiplied indefinitely through budding, grafting, or cuttings.

For these reasons, Scorza feels that although perennial fruit and nut crops have been relatively difficult subjects for gene isolation and transfer, these crops over time could reap the greatest benefits from such technologies.

This study also produced some new, interesting results on proteins involved in cold acclimation and dormancy.

"Dormancy is generally believed to be a prerequisite for cold acclimation in woody perennials. But we found that although the evergreen peach doesn't go dormant, it does increase its hardiness during the fall," says Rajeev Arora, a plant physiologist working at the Kearneysville lab.

This indicates, he says, that halting growth may not be an absolute requirement for cold acclimation.

"However, maximum hardiness attained by the deciduous trees was more than twice that of the evergreens," Arora says.

SCOTT BAUER

**Plant physiologist Rajeev Arora separates peach bark proteins for cold-hardiness studies. (K4686-1)**

The scientists found that two storage proteins, called 16 kD and 19 kD, accumulate to high levels in deciduous trees but not in evergreens.

"We're now trying to evaluate the role of these proteins in cold acclimation and dormancy," Arora says.

Accumulation of bark storage proteins is apparently regulated by day length. There's something in the tree's system that senses the change in day length. But the evergreen peach used as a parent in the study came from an area in Mexico where seasonal shifts in day length are minimal.

"This is really interesting," Arora says. "The evergreen peach may be missing that particular system in the tissues that senses the change in day length—which could account for its low levels of the storage proteins."—By **Doris Stanley,** ARS.

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# Natural Antifreeze for Underage Trees

Freezes over the past several years—which have slashed production in northern Florida as much as 98 percent—have caused the citrus industry to shift farther south in the state.

Cold-hardiness is an important characteristic in most crops grown in temperate zones. Therefore, supercooling—a natural protection from freeze damage—is as important in subtropical evergreen citrus as it is in deciduous temperate tree crops.

"We're taking a holistic, or whole plant, approach to preventing internal freezing of citrus tissues," says ARS plant physiologist George Yelenosky. "Instead of just looking at leaves, stems, buds, or flowers, we're using computerized sampling sensors and thermal regulators for whole trees in environmentally controlled rooms."

Although unable as yet to satisfactorily predict or manipulate supercooling to a set temperature, Yelenosky and colleagues have found that they can reinforce the process by spraying citrus leaves with abscisic acid and other compounds.

At the ARS Horticultural Research Laboratory in Orlando, Florida, Yelenosky, Heinz K. Wutscher, and Joseph C. Vu, now at Gainesville, have been using what they call "supercooling-stabilizers."

"These are simply blends of compounds that allow young, unhardened citrus trees to supercool more deeply and survive at around 22°F," Yelenosky explains.

Young trees treated with these compounds have survived for up to 2 hours what normally would be a lethal freeze without any injury or evidence of ice forming in the tissues.

Plant physiologist Michael Bausher is investigating the role of a newly found glycoprotein, GP-24. GP-24 was found in Poncirus, the most cold-hardy type of citrus, which is also deciduous. And in collaboration with the University of Florida, Bausher is studying unique proteins associated with citrus cold-hardening, called cold acclimation proteins.

We're taking a holistic, or whole plant, approach to preventing internal freezing of citrus tissues.

"We're also working on research to infuse antifreeze proteins (AFP2) found in arctic fish into citrus," says Yelenosky.

Orlando plant geneticist Randall P. Niedz says AFP2 could increase supercooling, reduce the amount of freezable water at a given temperature, and decrease the rate of ice spread in the tissues.

Scientists from the Plant Biotechnology Institute in Saskatoon, Saskatchewan, and Louisiana State University in Baton Rouge are collaborators in this research.

A type of inedible citrus has been shown to supercool at temperatures as cold as 5°F. Even its flowers can supercool and survive at 21°F; its callus, at 12°F.

In subtropical regions like Florida, this is important since temperatures in relatively short freezes seldom drop below 18°F, and they often remain above 21°F. Under these conditions, according to Yelenosky, it's possible that supercooling could be developed into a significant freeze-protection mechanism.

Like deciduous tree crops, citrus requires many hours of cool temperatures to harden. Ideally, those temperatures should be between 32°F and 50°F. About 500 continuous hours within this temperature range are needed before a severe freeze to prepare citrus for survival. In Florida, this ideal conditioning period is never assured and rarely achieved.

"Undoubtedly," says Yelenosky, "if we could adequately manipulate supercooling for citrus trees, we could not only save millions of dollars for Florida growers, but could also revolutionize freeze-management strategies for world agriculture."—By **Doris Stanley,** ARS.

*George Yelenosky and other scientists mentioned are at the USDA-ARS U.S. Horticultural Research Laboratory, 2120 Camden Road, Orlando, FL 32803. Phone (407) 897-7300, fax number (407) 897-7309.* ◆

BARRY FITZGERALD

**Live citrus being checked by plant physiologist George Yelenosky attest to the benefits of supercooling in this climate chamber freeze test. (K2551-16)**

# Safeguarding Winter's Food Supply

The frost is on the pumpkin and the fodder's in the shock. Late October sunshine adds a soft glow to brilliant patches of color splashed by oaks, maples, and beeches across the landscape. Autumn has come and it's harvest time.

But for farmers in the Sunshine State, it's planting time. More than half of the fresh fruits and vegetables eaten by Americans during winter months are grown in Florida.

To produce this bounty, Florida growers plant more than 730,000 acres of citrus and over 10 million acres of other crops and pasture.

"And when much of the country is worried about heating bills and snowy, slippery driving conditions, we're planting and harvesting tomatoes, snap beans, cabbage, celery, sweet corn, and squash," says Richard T. Mayer. "Here in Florida we grow a few crops that are shipped every month of the year." Mayer is head of the U.S. Horticultural Research Laboratory, the ARS research facility located in Orlando.

Mayer and his staff are looking for ways to keep food plentiful at economical costs. This can be done, he says, with research that will improve fruit and vegetable quality and protect crops from pests, diseases, and postharvest problems such as chilling damage during shipping or storage.

### Turning Up the Heat To Keep Cool

Supplying fresh produce for distant parts of the country means that maximizing its storage life is essential.

At the ARS Orlando lab, this responsibility falls to horticulturist Roy E. McDonald and staff.

"One problem with shipping fresh fruit and vegetables is that each commodity has its own ideal shipping temperature," McDonald says. "Since fresh produce is alive, it breathes. And without proper atmospheric conditions and refrigeration, shelf life diminishes, leaving the produce more susceptible to decay."

This means produce must be shipped at reduced temperatures. But low temperatures can cause chilling injury and abnormal ripening, both of which decrease quality.

"This creates a tough situation—a real double bind. What we really need is a way to prevent chilling injuries," says McDonald.

And as contradictory as it may sound, he is using heat to do that very thing. For some fruit, such as mangos, heat treatments also reduce decay, slow down ripening, and can be used as a quarantine treatment to control insect pests.

T. Gregory McCollum, an ARS plant physiologist, has been working with McDonald to make fruit more tolerant to lower storage temperatures. McCollum has come up with a high-temperature conditioning technique that improves the shelf life of mangos and could expand the market for that crop.

"Being tropical fruit, mangos get chilled at temperatures below 50°F. Rinds turn from pinkish-red to gray, sunken lesions appear on the skin, uneven ripening occurs, inner color and flavor development slow down, and the fruit becomes more susceptible to decay," he says.

McCollum transported mature mangos in an air-conditioned van from a commercial packinghouse to the ARS lab on the day the fruit was harvested. For 48 hours, he kept the mangos in a storage room at about 100°F. After 2 days, he decreased the temperature to 41°F—an appropriate shipping temperature—and left the fruit for 11 days.

"The mangos didn't look much different from the way they looked when they first came off the tree," says McCollum.

However, fruit in the control group that had not been subjected to high temperatures showed all the signs of chilling injury. "Heat preconditioning probably added a week to the test fruit's shelf life," McCollum says.

McCollum carried his research further. "Some growers may not be able to hold their produce at higher temperatures for 48 hours," he says. "So we took cucumbers from the field and instead of putting them in a high-temperature storage room, gave them a hot water bath."

For an hour the cucumbers sat in water heated to 108°F. Then they were chilled at 36°F for 2 weeks. McCollum says there was little rind pitting and tests showed few internal physiological changes.

Normally, cucumbers, which are very susceptible to chilling injury, are taken from the field, washed, precooled, and shipped at the temperature that is best for them. But if the heat conditioning protects them from chilling injury, they could be shipped in mixed loads with other commodities that require lower temperatures.

More than half of the fresh fruits and vegetables eaten by Americans during winter months are grown in Florida.

Chilling injury causes breakdown of the internal cell structure in fresh produce. But heat has been found to inhibit this cellular breakdown in cucumbers. McCollum's tests showed very few water-soaked areas in the cucumbers that had been soaked in hot water.

What goes on physiologically within the mangos and cucumbers to protect them from injury when they're hit with high, then low, temperatures?

According to McCollum, no one really knows. "We do know that when plants are exposed to nonlethal stress, they become more resistant to other types of stress."

Apparently, the stress of exposure to 100°F temperatures conditions the fruit and helps it to adjust, or acclimate itself, to later drops in temperatures during shipping.

McCollum says novel compounds called heat shock proteins exist, but they aren't present until plants are exposed to high temperatures. "We haven't researched this idea yet, but it is one of our hypotheses," he comments.

Plant physiologist Elizabeth Mitcham says heat can keep tomatoes from ripening and softening too quickly.

"Softening is an important aspect of ripening, but it causes tomatoes to be more susceptible to bruising and decay," she says.

Mitcham stored mature green tomatoes at 104°F for 4 days, then lowered the temperature to 70°F.

"After 10 days, treated tomatoes were still firm, with just a slight red color," she says. "But the untreated fruit was blood red and completely ripe."

The main reason fruit softens during ripening is that changes occur to the structure of the cell wall. These include a loss of sugars—particularly, galactose and arabinose.

But heat treatment inhibited the loss of these sugars, delaying cell wall modification and fruit softening.

SCOTT BAUER

**Entomologist Richard Mayer (left) and technician Mike Burkhart examine insect cell cultures that will be used for metabolic studies. (K4805-2)**

Tomatoes are usually picked mature green for later ripening in storage at temperatures between 57° to 60°F. To retard the ripening process, they must be chilled in the field, during transit, and in storage.

However, tomatoes are chilling-sensitive and cannot be stored below 55°F without injury. Therefore, low temperatures can't be used to greatly delay ripening.

The new heat treatment could cut tomato losses during shipping and also reduce low-temperature storage costs. "We need more time to work out the optimum time and temperature combinations," Mitcham says.

The Florida fruit and vegetable industry anxiously awaits more information on using heat treatments to prolong shelf life of fruits and vegetables.

"We're very supportive of this research and are particularly interested in ARS' work with mangos," says Craig A. Campbell, director of research and development for J.R. Brooks & Son in Homestead, Florida. Campbell says perishability and disease are the main problems they face in shipping mangos.

One of the biggest shippers of tropical fruit and vegetables in the United States, J.R. Brooks handles about 100 million pounds of fruit annually, including about 15 million pounds of mangos. The company imports fruit for U.S. consumers from 10 countries. *[This article was written before Hurricane Andrew severely damaged south Florida where Brooks is located.—Ed.]*

"Central and South American growers are very interested in shipping mangos to the United States to fill the market gap that can't be met by our own growers. But again, the problem is short shelf life," Campbell explains. "Slowing down ripening with heat treatments would greatly expand market potential for mangos."

SCOTT BAUER

**Entomologist Desmond R. Jimenez removes intercellular proteins from squash leaves that were caused by sweetpotato whitefly feeding. (K4803-2)**

## Major Milestones for the Horticultural Research Laboratory

**1930** - Released the Orlando tangelo to Florida nurseries. In 1990, growers harvested a crop valued at $10 million from 1,215,000 Orlando tangelo trees.

**1945** - Established fresh fruit maturity standards for marketing oranges and, in 1952, for grapefruit.

**1972** - Introduced a family of microhymenoptera parasites to control scale insects and, in 1979, helped Florida state scientists (who had originally used a chemical control) identify a different family of the parasites that control citrus blackfly. Because of this work, neither scale nor blackfly is considered a threat to Florida citrus.

**1973** - Designed the fiberboard box now used for exporting all citrus fruit.

**1974** - Released swingle citrumelo citrus rootstock to nurseries. Determined its resistance to citrus nematodes in 1981 and its tolerance to citrus blight in 1988. Swingle now makes up 51 percent of Florida's nursery stock.

**1979** - Helped citrus industry design a semirigid insulation wrap that protects young citrus trees from freezing. Now used extensively by citrus growers.

**1982** - Developed cold treatment for grapefruit that allowed export to Japan and other quarantine countries and states. In the same year, developed heat treatments to disinfest citrus nursery trees of nematodes and for specific nematode problems in 1985.

**1989** - After 26 years of breeding, introduced Ambersweet, the first cold-hardy orange. Growers have already planted at least 10 million trees.

**1990** - Found three nematode species that biologically control citrus root weevil. Growers throughout Florida are now using the nematodes, which are being produced commercially.

**1990** - Developed a test to rapidly diagnose citrus blight, a disease that kills about half a million citrus trees annually.

**1992** - Patented a practical, reliable test to differentiate strains of citrus tristeza, a virus that can kill trees.

Since his company grows, packs, and/or ships 40 different tropical fruits and vegetables, Campbell hopes that the treatment will be tried on other commodities in addition to mangos, cucumbers, and tomatoes.

### Before the Reaping—A New Pest Threatens Quality

ARS scientists are actually thinking about the quality of fruits and vegetables long before they are ready for shipping—in fact, long before harvest.

A new insect pest that has recently appeared in Florida is causing concern because it has the potential to devastate many vegetable, fruit, and ornamental crops.

First sighted in this country in southern Florida in 1990, *Thrips palmi* has spread into eight Florida counties.

"The pest is damaging in all stages of its life cycle," says ARS Orlando lab director Richard Mayer, "even the winged adult." Called a rasping insect, *T. palmi* drags its sharp mouthparts across leaves, growing tips, flowers, or fruit to cause them to ooze fluid, which it feeds on. Leaves and fruit of damaged plants are bronzed, stunted, or severely scarred.

SCOTT BAUER

**As the first step in isolating proteins that bind plant compounds in the larva, entomologist Jeffrey Shapiro collects hemolymph (blood) from root weevils. (K4806-4)**

To find a solution, ARS joined forces with scientists from the University of Florida, Florida State Department of Agriculture, and USDA's Animal and Plant Health Inspection Service, and with growers, processors, and commodity groups.

There is no wholly satisfactory chemical control for the new pest. Field trials using several different pesticides and combinations of pesticides have been tried.

"Although now confined to Florida, *T. palmi* could spread through the Gulf Coast states and parts of the Southwest and could easily infest greenhouses in the North," Mayer says.

Growers have found it on eggplant, green and wax beans, jalapeno and bell peppers, potatoes, okra, zucchini, cucumbers, passionfruit, mangos, and several ornamentals.

Working with University of Florida scientists, ARS entomologists William J. Schroeder and Kim Hoelmer are rearing an experimental colony of *T.*

*palmi* on a variety of vegetable plants at Apopka, Florida.

"We're using this colony to evaluate natural controls such as fungi and other disease-inducing organisms, nematodes that might be introduced into the soil, and possible predators and parasites," Schroeder says.

ARS is also exploring potential biocontrol agents at overseas laboratories. Candidate biocontrols could be tested at ARS quarantine facilities in the United States.

Also called melon thrips, *T. palmi* is thought to be native to the Malaysian-Indonesian region. In addition to Japan, it is found in Hawaii and the Caribbean.

According to Hoelmer, the female lays hundreds of eggs in leaf tissues. In 11 to 24 days, these microscopic white eggs become mature thrips.

"One of the major problems with this pest is that populations grow so rapidly they displace other, less damaging insects," Hoelmer says.

Jack Lee, president of Spectrum Farms, Inc., grows vegetables on about 700 acres in Homestead. "This pest is as bad as—if not worse than—any freeze that has ever hit Florida," he says.

Crop loss and extra chemical spraying have already cost him well over $150,000.

Grower D. Webster Williams, who raises potatoes for the fresh market, says, "I've never seen anything like this in all my 41 years of farming." Webster lost about a fourth of a 50-acre potato field to *T. palmi* last year.

Since crop production can be year-round in Florida, growers are anxious for researchers to identify possible ways to fight this devastating pest.

Working with cooperators, ARS has identified several areas of needed research, which include controlling the pest, treating affected produce, and searching for host plant varieties that can resist *T. palmi* infestation.

## Heading Off Citrus Diseases and Nematodes

A major problem in keeping citrus and other crops healthy is identifying diseases and other problems before they destroy crops.

Especially potent or severe strains of citrus tristeza virus (CTV) pose a serious problem to fruit growers. These strains of the virus can kill trees within a matter of weeks after the first symptoms appear.

Surprisingly, mild strains of the virus may actually protect trees from infection by severe strains. But it has been impossible until now to differentiate between types of CTV.

"We've developed a quick, practical, and reliable diagnostic test using a monoclonal antibody," says David T. Kaplan, who leads ARS subtropical plant pathology research at Orlando.

SCOTT BAUER

**Plant physiologist Greg McCollum immerses cucumbers in a heated water bath that paradoxically makes them more tolerant of cold during shipping. (K4799-4)**

The antibody was developed and patented by plant pathologist Stephen M. Garnsey and former ARS research associate Tom Permar.

"The State of Florida already uses our testing method to certify that budwood used for propagating new citrus groves is free of severe, decline-inducing strains of CTV," Kaplan says. It is also used to identify severe strains in Central America and the Caribbean Basin that, if accidentally brought into the United States, could threaten our citrus. And the test can identify beneficial protective strains of CTV.

In another research approach, Kaplan says, "We've identified a gene in a citrus relative that would make citrus resistant to CTV." ARS pathologists and horticulturists are working to breed this gene into new scion and rootstock varieties.

Citrus production is also hampered by parasitic nematodes—microscopic worms that feed on and destroy roots, lessening plant vigor and productivity.

Kaplan says that nematodes cause $4 billion in lost revenue annually worldwide. These losses will likely increase in the United States, since most nematicides previously used in citrus groves have been banned for environmental concerns.

Some citrus rootstocks resist nematodes, but some nematodes can overcome this resistance. It is almost impossible to identify these extra-aggressive nematodes. But Kaplan is working with ARS plant breeders at the Orlando lab to develop rootstocks with natural resistance that even the toughest nematode can't overcome.

## Exploiting Natural Plant Defenses

Protecting crops from pests and diseases and improving the quality of fruits and vegetables are important parts of the diverse Orlando research plan.

So is the research of entomologist Jeffrey P. Shapiro, who studies the

SCOTT BAUER


**Entomologist Bill Schroeder watches the rasping action of a feeding *Thrips palmi* on a bean leaf. (K4800-8)**

interactions between insect pests and the natural chemicals present in their host plants.

"This interaction is vital to both the plant's self-defense and the insect's survival," he says.

According to Shapiro, the successful exploitation of natural products against insects is still in its infancy. "Future success hinges on discovering, breeding, and genetically engineering effective deterrent compounds into host plants."

He has isolated and is now identifying several potential defensive compounds from citrus. And he advocates manipulation of insect defenses as well.

For example, "We've found certain blood proteins in root weevil larvae that are responsible for absorbing and transporting plant compounds. Therefore, disrupting or synergizing these proteins could lead to new strategies to control the weevil," he explains.

Along with ARS entomologist Desmond Jimenez, Shapiro is also studying plant proteins. They've found proteins in squash and pumpkin that appear or disappear when attacked by some strains of whitefly.

"Whether these proteins help the plant defend itself against the pest or not, their genetic control may yield clues to natural defensive responses," says Shapiro.

He predicts that the genetic engineering of plant defenses, combined with biological and cultural controls, will eventually replenish our declining arsenal of synthetic insecticides.—By **Doris Stanley**, ARS.

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# Food Poisoning Cases Linked by DNA Fingerprints

The killer grabbing headlines across the United States was one that even the resourceful minds of public health "detectives" couldn't track down.

But ARS researchers at the National Animal Disease Center in Ames, Iowa, managed to "fingerprint" the culprit implicated in the three worst cases of food poisoning known to occur in North America.

Just 2 years ago, a 6-year-old child from northern California was diagnosed with meningitis, an infection of the membranes surrounding the brain and spinal cord. Medical specialists suspected his infection was caused by food poisoning.

Could the culprit be *Listeria monocytogenes,* the same bacterium responsible for 142 listeriosis cases and 48 deaths during the 1985 Southern California epidemic involving Mexican-style cheese?

The child had eaten such a Mexican-style soft cheese, and state health authorities wanted to know if bacterial isolates matched isolates from the cheese and from the factory where the cheese was made.

Enter ARS microbiologist Irene V. Wesley and co-researchers at the NADC. They were the only scientists in the country using restriction enzyme analysis to check the isolates involving *L. monocytogenes* from these three outbreaks.

Says Wesley, "We 'fingerprinted' isolates from each of the three big listeriosis outbreaks—Canada (1981), New England (1983), and California (1985). Most isolates recovered from each of the outbreaks showed a DNA pattern characteristic of that episode.

"Now we can refer to the New England DNA pattern, the California DNA pattern, and so on."

The method is simple and can be used by lab technicians and public health safety workers to track the spread of *Listeria* from the food-making environment to the food product and on to the human patient.

In analyzing the *Listeria* isolated in the northern California case, the researchers found that the isolates recovered from the patient, the suspect cheese, and the cheese factory all matched.

"Not only were they identical to each other, but the DNA pattern from that isolated case matched the profile of *L. monocytogenes* recovered from the 1985 southern California epidemic," adds Wesley.

The victims of the 1985 California epidemic were mainly pregnant Hispanic women. Wesley says the likely source of contamination was raw milk that had become mixed with pasteurized milk during the cheese-making process.

Two years earlier, in 1983, milk from a major grocery chain was believed to be implicated in 17 deaths in Massachusetts. This incident prompted the U.S. Food and Drug Administration (FDA) to ask NADC researchers to assist in a study of the

BRUCE FRITZ

**Microbiologist Irene Wesley prepares to take a blood sample from a cow infected with *Listeria monocytogenes.* (K4807-1)**

pasteurization process and its effectiveness in controlling *Listeria.*

In this joint project, NADC researchers established a herd of *Listeria*-infected dairy cows in Ames, Iowa. *Listeria*-contaminated raw milk from the herd was shipped to the FDA laboratory in Cincinnati, Ohio, for pasteurization. FDA scientists confirmed that pasteurization kills *L. monocytogenes* in milk.

Wesley examined 14 strains of *L. monocytogenes* from patients and raw milk recovered from the 1983 New England outbreak.

According to Wesley, it is important to know that "Not all strains of *Listeria* are deadly. The DNA fingerprints of *Listeria* that we examined indicated that milk was not responsible for the fatal infections during the 1983 epidemic." But to this day, the cause of that outbreak remains a mystery.

The last of the three major epidemics Wesley studied occurred in 1981. *Listeria* was traced to coleslaw served in Nova Scotia that caused 41 cases of listeriosis and 14 deaths. Epidemiologists speculated that the cabbage had been grown in fields fertilized with manure from *Listeria*-infected sheep.

## Finding and Identifying Harmful Strains

DNA fingerprinting can be completed in less than a week. And time is critical when dealing with potentially contaminated food products.

"The sooner a suspect pathogen is found, the faster food processors can recall products and prevent their widespread distribution throughout the country," says Wesley.

*L. monocytogenes* kills one out of three people in whom clinical disease becomes evident. Found in soft cheeses, milk, delicatessen food, and undercooked meat, poultry, and seafood, *L. monocytogenes* sickens about 1,850 Americans yearly, according to a recent report from the Centers for Disease Control (CDC) in Atlanta, Georgia.

Where does this killer bacterium lurk? In addition to isolating it from both raw and processed foods, scientists have also found it in the soil, in vegetation, and in animals.

"But finding *Listeria* in foods doesn't always mean people will get infected and develop listeriosis. In fact, some people may have a natural immunity to infection," says Wesley.

"Most healthy people," she says, "needn't be alarmed about *Listeria* food poisoning because fatalities usually occur within special high-risk population groups."

These include the young, the elderly, pregnant women and the unborn, and people with weakened immune systems. In pregnant women, listeriosis causes intrauterine infections that can lead to miscarriages and stillbirths.

Listeriosis patients have mild, flulike symptoms that can start as soon as 48 to 72 hours—or as late as 4 to 8 weeks—after eating contaminated food. People feel like they're coming down with the flu, so they usually don't seek medical help.

"But once full-blown symptoms develop, listeriosis has a higher mortality rate (25-40 percent) than any other foodborne disease. The mortality rates for *Salmonella* and *Campylobacter* are less than 1 percent," says Wesley.

The CDC recently reported 165 confirmed cases of human listeriosis in California, Georgia, Tennessee, and Oklahoma between November 1988 and 1990.

To date, Wesley has participated in food safety studies with FDA,

### How Can You Protect Yourself and Your Family?

While you can't see, taste, or smell *Listeria* in food, a few simple, common sense food preparation precautions will help prevent all kinds of foodborne illnesses. They are:

Don't drink raw milk.

Wash hands thoroughly both before and after preparing foods.

Use a plastic, dishwasher-safe cutting board—not wood.

Wash countertops, cutting board, and any utensils that have come in contact with raw meat, vegetables, or seafood.

Don't prepare, cut, or carry raw meat, poultry, and seafood on the same surface or plate that you later use for cooked foods or other foods.

Don't let juices from raw meat drip on other foods.

Rinse and scrub raw vegetables thoroughly.

Cook meat, poultry, and seafood thoroughly to kill microorganisms. USDA recommends that beef be cooked to an internal temperature of 160°F at its thickest, poultry to 185°F, and seafoods until they flake and easily break apart.

Refrigerate foods promptly after a shopping trip. Even though *L. monocytogenes* can grow while foods are refrigerated, it's wise to keep them cold for as long as possible before cooking or serving.

CDC, and USDA's Food Safety and Inspection Service. Also, various state departments of health have sought her expertise in solving isolated cases of *Listeria* food poisoning.—By **Linda Cooke,** ARS.

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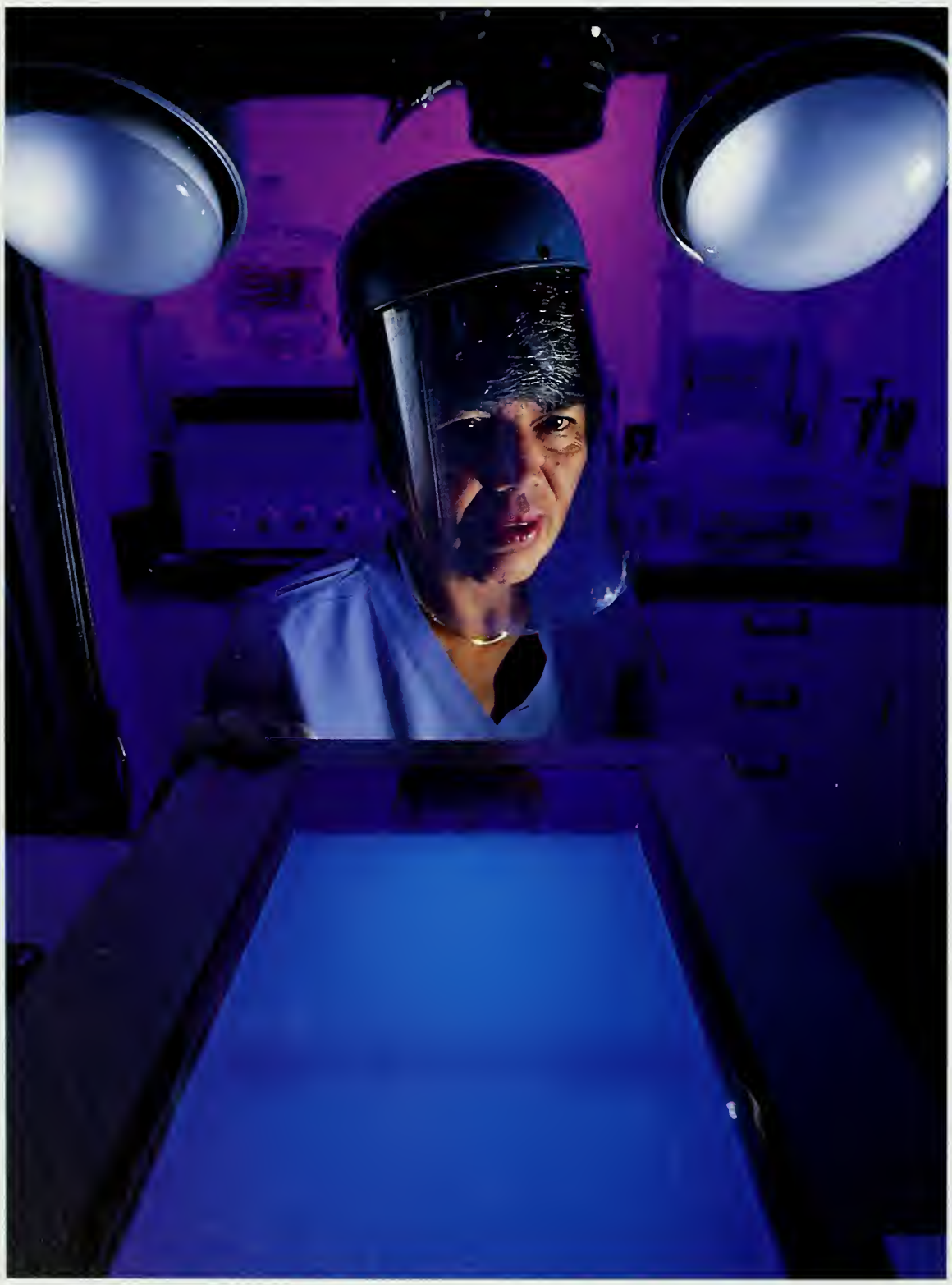

**To track the spread of *L. monocytogenes* from the suspected food source to the human patient, microbiologist Irene Wesley analyzes bacterial DNA patterns.**

### In What Foods Have *L. Monocytogenes* Bacteria Been Found?

Dairy Products: cheese, milk, ice cream, ice milk, novelty ice cream products (for example, ice cream bars)

Meats: raw beef and poultry

Processed Meats: hot dogs, luncheon meats, prosciutto

Seafood: imported shrimp, imported and domestic crabmeat, mackerel, and pollack

Vegetables: cabbage

### Who Are the People in the Listeriosis High-Risk Group?

People in the following categories should get medical attention if they suspect an *L. monocytogenes* infection:

Pregnant women

Elderly

Individuals with depressed immune systems caused by such conditions as: AIDS, alcoholism, cancer, cirrhosis, diabetes, drug abuse, ulcers, ulcerative colitis, or extended use of steroid medications

# AGNOTES

## Pill or Pesticide? It's Both!

For people suffering gout's excruciating joint pain, allopurinol is a magical pain reliever.

For cockroaches trying to build a robust population in your home, the drug is a major pain.

That's because Agricultural Research Service studies show that the drug prevents German cockroach reproduction, obliterating entire localized cockroach populations, says Daniel R. Suiter, a graduate student working at the agency's Medical and Veterinary Entomology Laboratory in Gainesville, Florida.

The drug relieves gout pain by preventing a buildup of crystallized slivers of overabundant uric acid that form around a gout sufferer's joints.

Allopurinol chemically resembles a uric acid precursor, so enzymes needed to produce—or overproduce—the culprit crystals are instead bound up by the drug.

In cockroaches, allopurinol prevents the manufacture of uric acid in the same way. But inseminated female cockroaches need uric acid for development of embryos within the fertilized eggs. Without the crucial acid, females simply can't reproduce.

Suiter and Richard Kramer, formerly at the Gainesville lab, in collaboration with University of Florida entomologist Philip G. Koehler, have conducted allopurinol tests on thousands of German cockroaches. When the scientists offered rat chow laced with the drug and rat chow without it, the cockroaches ate both equally well. But in 4 to 6 weeks, the population feeding on the rat chow with allopurinol died off, Suiter says.

The scientists found that the dose that best and most efficiently reduces roaches is only one-tenth of a percent allopurinol. ARS and the University of Florida share a patent on this new allopurinol use. So far, several commercial pesticide companies have expressed interest.

Additional studies may be needed, however, to receive U.S. Environmental Protection Agency approval of a new use for the allopurinol.—By **Jessica Morrison Silva,** ARS.

*Daniel R. Suiter is at the USDA-ARS Medical and Veterinary Entomology Laboratory, 1700 SW 23rd Drive, P.O. Box 14565, Gainesville, FL 32604. Phone (904) 374-5910, fax number (904) 374-5818.* ◆

## Bagging Boosts Banana Quality, Profits

Banana growers in Puerto Rico can boost their net profits by $1,350 an acre simply by covering growing fruit with special bags, a 40-month study shows.

Using perforated polyethylene bags—either treated with insecticide or untreated—increased production by 9,407 pounds per acre (an increase of 7 percent) during the study conducted in Puerto Rico by ARS and the University of Puerto Rico.

"The bags stop mites from damaging the fruit surface and create a constant temperature so fruit reaches the mature green stage 1 to 2 weeks faster," says Heber Irizarry, an ARS horticulturist in Mayaguez, Puerto Rico.

The study's overall goal, Irizarry says, was to develop management practices to improve banana quality. Only 23 percent of Puerto Rico's 93,500-ton banana crop is sold as ripe fresh fruit. One reason: Mite and blemish damage make many bananas suitable only for cooking. Also, many growers still use the long-standing practice of planting banana and coffee plants side by side, so the taller banana trees provide shade for the coffee plants. But that makes it difficult to properly manage the banana bunch.

"By improving fruit quality, growers in Puerto Rico could ship bananas to the United States, which now imports most of its bananas from Costa Rica, Honduras, and Ecuador," says Irizarry.

Those countries use bags in banana production, but Irizarry's is the first study of the method in Puerto Rico.—By **Sean Adams,** ARS.

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## Pears Wax Hot or Cold

A pear's tender, smooth skin can easily tear as it rolls down a conveyer belt in the packinghouse. To prevent this belt burn damage, packers traditionally coat fruit for U.S. markets with a light covering of an all-natural wax.

The most commonly used wax—carnauba—comes from Brazilian palm trees. Without wax, many pears would be ruined, because even a tiny tear or nick in the skin will quickly turn into a soft, mushy brown spot.

Hot air blown over the pears is typically used to dry the wax. But ARS horticulturist Stephen R. Drake found that cold air—at a chilly 32°F—dried the wax just as quickly and offered other benefits to boot.

"For one thing, it takes less energy to use cool air," says Drake. The hot air temperature reaches 140°F—about the same as the highest setting on the average hair blowdryer.

The hot air also warms the pears slightly. Compared to cool-dried pears, hot-dried pears take almost a full day longer to reach an equilibrium with the temperature in cold storage rooms where they're stored until shipping. So again, the cool air treatment is more energy efficient.

Drake also found that waxed, cold-dried pears stayed firmer longer than those that were waxed and dried with hot air. That's an advantage for pears that stay in storage for extended periods before being shipped to grocers.

Although he used only d'Anjou pears for the study, Drake expects other varieties would show similar results.

California and Washington grow most of our nation's pears. Bartletts and Boscs, along with d'Anjous, are the most popular varieties. Fresh pears have only about 60 calories each and are a fair source of fiber and potassium.—By **Julie Corliss**, ARS.

*Stephen R. Drake is in the USDA-ARS Physiology and Pathology of Tree Fruit Research Unit, Tree Fruit Research Laboratory, 1104 North Western Ave., Wenatchee, WA 98801. Phone (509) 664-2280, fax number (509) 664-2287.* ◆

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# World Food Prize Winners

**F**ormer ARS scientists Edward F. Knipling and Raymond C. Bushland have been named the 1992 recipients of The World Food Prize.

Now retired, the two were honored for joint efforts begun more than 50 years ago that gave the world a new and environmentally friendly means to control insect pests. That research has helped sustain vast sources of food sorely needed by the growing world population. It has also prevented untold suffering and losses in both wildlife and human populations.

Says John Ruan, chairman of The World Food Prize Foundation, "This team of visionary scientists dedicated years of relentless scientific research, and cooperative effort with U.S. and world organizations, to improve the world's food supply and human health."

Norman Borlaug, chairman of The World Prize Foundation selection committee, notes especially the ecological compatibility of this innovative and sound insect control method.

Specifically, the award acknowledges development of what is called the sterile insect technique. This technology was originally designed to overcome the devastation to livestock caused by the screwworm, a pest that in its larval form consumes the living flesh of mammals. The biological technique uses no chemicals and does not affect other nontarget insect species.

Instead, it relies on the periodic release of healthy male flies sterilized by exposure to radiation and their mating with native females. Such unions produce no offspring. In time, given the release of sufficient numbers of sterile males to overwhelm native screwworms, the population within a geographic area can be eliminated. (For more details, see *Agricultural Research,* July 1992, pp. 6-7.)

**Edward F. Knipling**

**Raymond C. Bushland**

This sterile fly technique has been used successfully to eradicate screwworms from the United States, Mexico, Belize, Guatemala, and—most recently—Libya. Control programs are currently under way in Honduras and El Salvador.

Sterile insect technology has also proved effective in controlling the tsetse fly, Mediterranean fruit fly, melon fly, pink bollworm, codling moth, and onion fly. Further advancement and adaptation of this technology offer great hope to farmers and consumers.

The World Food Prize is the largest award given for accomplishment in food and agriculture. Knipling and Bushland will each receive a commemorative sculpture and share a cash award of $200,000.